Mitigating Radiation-induced Bone Loss via Dietary Modulation of Inflammatory Cytokines

Completed Technology Project (2016 - 2019)

Project Introduction

Proposal Summary: Astronauts traveling to Mars or other planetary surfaces will be exposed to low doses (up to 2 Gy) of high-energy radiation particles that compose galactic cosmic radiation (GCR). A growing body of literature documents a rapid acceleration of bone resorption activity and some suppression of bone-forming cells after exposure to even very low doses of space-relevant radiation. One of the key mechanisms proposed for these changes is an increase in pro-inflammatory cytokines like TNF-a. We already know that International Space Station (ISS) crew members consuming a diet rich in omega-3 Fatty acids (FAs) experience smaller reductions in bone mineral density over 6-month missions than do fellow astronauts consuming a diet low in these FAs. This project proposes to investigate the impact of consuming a diet high in omega-3 FAs on radiation-induced pro-inflammatory cytokines. Hence, this simple dietary intervention may provide a low-cost, low-risk means of countering the harmful effects of radiation on bone integrity.

A currently funded NASA Space Biology study based at Texas A&M (Principal Investigator (PI): Dr. Nancy Turner) will expose multiple cohorts of mice at Brookhaven National Laboratory's NASA Space Radiation Laboratory (NSRL) facility to space relevant doses of high energy iron particles) over the next 12 months. Her project will determine the selective impact of radiation on radio-sensitive intestinal stem cells in two sets of animals: those consuming a corn oil-supplemented diet simulating the average American diet and those consuming a diet supplemented with fish oil, which elevates dramatically omega-3 FA intake. The PI's current collaborations with Dr. Turner and our laboratories' close proximity have enabled an unparalleled tissue sharing opportunity.

We propose to collect both long bones and serum from these animals to test our over-arching hypothesis: a diet high in omega-3 FAs will mitigate radiation-induced bone loss by reducing the generation of inflammatory cytokines in bone tissue. The study offers the possibility of comparing responses to 3 different space-relevant doses (0.1, 0.25, and 0.5 Gy) of 56Fe at 1 Gev/nucleon, along with a gamma reference group (0.25 to 2 Gy). Analyses of specimens collected at 12 hours post-exposure will focus on changes in pro-inflammatory cytokines in serum and in bone cells called osteocytes, which in turn signal to both bone-forming and bone-resorbing cells. The two later post-irradiation time points proposed (4 wk and 8 wk) will enable us to determine early and late effects on bone cell activity and bone structural integrity, important since the current data base on time course of bone alterations post-exposure is sparse.

Significance : Given that the expensive NSRL (NASA Space Radiation Laboratory) live animal experiments are already funded, this project can yield a wealth of new data important to minimizing fracture risk for exploration class missions at minimal extra cost to NASA. With this project we will

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Anticipated Benefits

With this project we will generate a comprehensive assessment of the impact of 3 doses of high-energy iron particles on bone integrity, at 3 dose levels and at 3 time points post-irradiation. Comparisons with gamma-irradiated groups at comparable doses will allow for RBE (relative biological effectiveness) determinations; sham irradiated animals will be sacrificed at the same time points. After just one year, we will have proof-of-concept and feasibility testing completed for a high omega-3 FA diet as a countermeasure to radiation-induced bone loss, and interesting science data on the role of osteocytes in the pro-inflammatory response to radiation. Should it prove successful, this countermeasure is ready for immediate operational implementation, as omega-3 fatty acid-rich diets and/or supplements are available now to ISS crew. These data will also be relevant to clinical patients undergoing radiotherapy and patients with chronic inflammatory disease (e.g., inflammatory bowel disease), all of whom experience significantly elevated fracture rates.

Organizational Responsibility

Responsible Mission Directorate:

Space Operations Mission Directorate (SOMD)

Lead Center / Facility:

Johnson Space Center (JSC)

Responsible Program:

Human Spaceflight Capabilities

Project Management

Program Director:

David K Baumann

Project Manager:

Peter Norsk

Principal Investigator:

Susan A Bloomfield

Co-Investigators:

Harry A Hogan
Nancy D Turner

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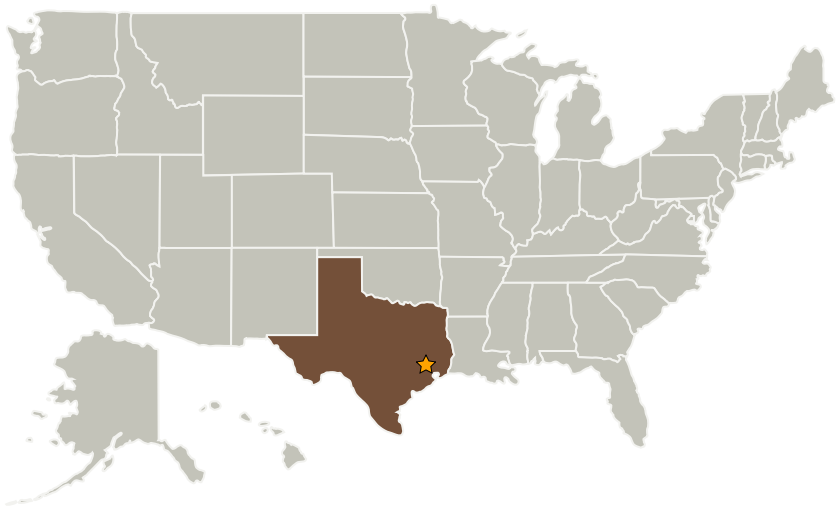
Primary U.S. Work Locations and Key Partners



Organizations Performing Work	Role	Type	Location
★Johnson Space Center(JSC)	Lead Organization	NASA Center	Houston, Texas
Michigan State University	Supporting Organization	Academia	East Lansing, Michigan
Texas A & M University-College Station(Texas A&M)	Supporting Organization	Academia Hispanic Serving Institutions (HSI)	College Station, Texas

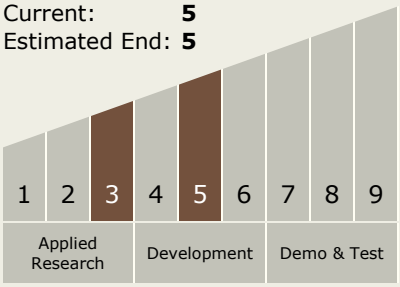
Primary U.S. Work Locations
Texas

Project Transitions

December 2016: Project Start

Technology Maturity (TRL)

Start: **3**
Current: **5**
Estimated End: **5**



Technology Areas

Primary:

- TX06 Human Health, Life Support, and Habitation Systems
  - TX06.5 Radiation
    - TX06.5.2 Radiation Mitigation and Biological Countermeasures

Target Destinations

The Moon, Mars

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August 2019: Closed out

Closeout Summary: Experimental Approach and Methods: Bones and serum collected from animals exposed to 56Fe and gamma radiation by the parent protocol at multiple doses (56Fe: 0.1, 0.25, and 0.5 Gy of 1000 MeV/n , 25 cGy/min at Brookhaven National Lab; gamma X-ray radiation: 0.2, 0.5, and 2 Gy, at Texas A&M) and time points (12 hr, 4 wk, 8 wk post-exposure) were stored by appropriate procedures to enable the proposed outcomes detailed below. One half of all animals were fed a corn oil-cellulose (COC) and the other half a fish oil-pectin (FOP) diet to test the impact of an "anti-oxidant" diet on inflammatory changes following irradiation. Tissues collected at 12 hours post-radiation exposure were assessed only for changes in serum TNF-a, serum TRAP5b (a marker for osteoclast number and therefore resorption activity) using commercial ELISA assay kits, and by immunohistochemical (IHC) staining for TNF-a and sclerostin (see Metzger et al., 2017 for methods) in metaphyseal bone osteocytes. Assessment of %-positive osteocytes for select proteins is, we believe, more useful than assessing altered gene expression of homogenized bone tissue, since the latter cannot confirm that increases in mRNA result in more protein product, nor is it localized to any one cell type. Any changes in bone structure would be too small to detect at this early time point. Tissues collected at 4 and 8 weeks post-radiation exposure were assessed for changes in serum TNF-a and IHC staining for osteocyte TNF-a and sclerostin [in R distal femur] and, in addition, by 1) micro-CT (in consultant Dr. Larry Suva's laboratory, TAMU Dept. of Veterinary Physiology & Pharmacology) for distal femur bone structural properties; 2) histomorphometry of distal femur for evidence of bone formation/ resorption activity, using an epifluorescent microscope interfaced with a CCD camera and OsteoMeasure software; and 3) [not yet completed] mechanical testing of mid-shaft tibiae and L femoral neck, using a desktop Instron device. Earlier annual reports included data on male mice; since very few significant effects were observed in those males, we proceeded to focus only on female mouse data. Hence, all results discussed below are for female mice only Statistical analyses were performed on all outcome measures using a two-way MANOVA (factors = diet, dose) with each time point and ion species analyzed separately and an alpha level of 0.10 set a priori. We believe using this higher p value threshold is appropriate for this project, given the very low n's in some groups and the exploratory nature of these experiments, erring on the side of inclusiveness. Results: (organized by Specific Aims) Specific Aim 1: Determine alterations in bone structural integrity (density, geometry, microarchitecture, mechanical properties); bone cell activity; serum TNF-a, and osteocyte TNF-a and sclerostin, in mice exposed to low dose HZE (high energy particle) radiation. • Hypothesis 1: An early up-regulation of serum TNF-a and TNF-a in osteocytes, after radiation exposure will be associated with increased resorption and decrements in bone structural integrity observed 4 and 8 weeks later; increased osteocyte sclerostin by 4 weeks will be associated with declines in bone formation activity. Little of our original working hypothesis about radiation exposure effects in our control (COC-fed) mice is borne out by our results. We do not observe any significant decrements in bone structural integrity, except for mice at 8 weeks after gamma exposure, when some significant declines were observed in distal femur cancellous bone mass indices (%BV/TV and vBMD) and in trabecular connectivity. The only group to exhibit any change in our bone formation index (% osteoid surface/total surface) were 56Fe–exposed mice after 8 weeks; this suggests that if we had tracked these mice for a longer time, we might ultimately observe a corresponding decline in cancellous %BV/TV. We found no evidence for an early pro-inflammatory response as measured by serum and osteocyte TNF-a (%+Ot.TNF-a), but rather a very late decrease in serum TNF-a 8 weeks after gamma exposure. Sclerostin, a negative regulator of bone formation, declines a small amount in cortical bone osteocytes 4 weeks after 56Fe exposure; IGF-I, a positive regulator of bone formation, appears to be slightly upregulated by 56Fe exposure in both cortical and cancellous bone compartments. Specific Aim 2: Determine impact of a diet high in omega-3 fatty acid content on osteocyte TNF-a and decrements in bone structural integrity after exposure to low dose HZE radiation. • Hypothesis 2: Mice consuming a high omega-3 FA diet will exhibit reduced serum TNF-a; reduced sclerostin and TNF-a in osteocytes; and attenuated decrements in bone structural integrity after radiation exposure. Cited below are relevant results when there was a significant main effect of diet and, in particular, a diet by dose interaction. We originally hypothesized that consumption of a diet high in omega-3 fatty acids (FOP) would result in a diminished increase in serum and osteocyte TNF-a, and in osteocyte sclerostin, which would explain mitigated decrements in structural integrity, following radiation exposure. The dietary impact was most clear at the 4- and 8-week time points for serum TNF-a values; following 56Fe exposure, serum TNF-a was indeed lower in FOP-fed mice. Intriguingly, a different effect occurred following gamma exposure, when FOP-fed mice at 8 weeks exhibited on average much higher serum TNF-a values than did COC-fed mice. At both these time points weeks removed from the time of irradiation, increasing doses of radiation either had no effects or resulted in declines in serum TNF-a values. However, when examining %osteocytes positive for TNF-a, the FOP-fed mice exhibit the same or slightly higher %+Ot.TNF-a values in both gamma- and 56Fe-exposed mice. Hence, the presence of TNF-a in these important regulatory bone cells does not track well with serum TNF-a. The most significant differences in bone structural integrity were observed in mid-shaft cortical bone diameters and the connectivity density in the cancellous compartment of the distal femur. Cortical bone diameters (but not area, or thickness of the cortical shell) at 8 weeks following 56Fe exposures were generally larger at all 56Fe doses in

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Stories

Abstracts for Journals and Proceedings
(https://techport.nasa.gov/file/64203)

Abstracts for Journals and Proceedings
(https://techport.nasa.gov/file/64205)

Abstracts for Journals and Proceedings
(https://techport.nasa.gov/file/64204)

Abstracts for Journals and Proceedings
(https://techport.nasa.gov/file/64206)

Articles in Peer-reviewed Journals
(https://techport.nasa.gov/file/64202)

Project Website:

https://taskbook.nasaprs.com